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Authors
Beemelmanns, Christine
Woznica, Arielle
Alegado, Rosanna A
et al.

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Synthesis of the Rosette-Inducing Factor RIF-1 and Analogs

Christine Beemelmanns,[†,§] Arielle Woznica,[‡] Rosanna A. Alegado,[‡,∥] Alexandra M. Cantley,[†] Nicole King,[*,‡] and Jon Clardy[*,†]

[†] Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115, United States

[‡] Howard Hughes Medical Institute and Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, United States

Supporting Information

ABSTRACT: Studies on the origin of animal multicellularity have increasingly focused on one of the closest living relatives of animals, the choanoflagellate *Salpingoeca rosetta*. Single cells of *S. rosetta* can develop into multicellular rosette-shaped colonies through a process of incomplete cytokinesis. Unexpectedly, the initiation of rosette development requires bacterially produced small molecules. Previously, our laboratories reported the planar structure and femtomolar rosette-inducing activity of one rosette-inducing small molecule, dubbed rosette-inducing factor 1 (RIF-1), produced by the Gram-negative Bacteroidetes bacterium *Algoriphagus machipongonensis*. RIF-1 belongs to the small and poorly explored class of sulfonolipids. Here, we report a modular total synthesis of RIF-1 stereoisomers and structural analogs. Rosette-induction assays using synthetic RIF-1 stereoisomers and naturally occurring analogs defined the absolute stereochemistry of RIF-1 and revealed a remarkably restrictive set of structural requirements for inducing rosette development.

Multicellularity, the transition from a unicellular to a multicellular organism, evolved at least 25 times within eukaryotes, but it evolved only once in the animal lineage.[1] Choanoflagellates, the closest living relatives of animals, have emerged as important model organisms for reconstructing the transition to multicellularity.[2] Choanoflagellate cells have a spherical to prolate spheroid cell body and an apical collar of microvilli surrounding a single flagellum (Figure 1)[3] that resembles the feeding cells (choanocytes) of sponges.

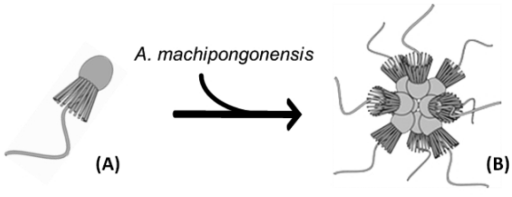

Figure 1. Morphogenesis of the choanoflagellate *S. rosetta* upon exposure to the prey bacterium *A. machipongonensis*: (A) unicellular slow swimmer and (B) multicellular colonial rosette form (drawing: courtesy of Mark Dayel).

Undulation of the apical flagellum generates water currents that sweep bacteria against the microvillar collar, where they are trapped and ultimately phagocytosed. One species of choanoflagellate, *Salpingoeca rosetta*, exhibits both free-living and multicellular colonial forms called rosettes; and this transition provides the basis of our study (Figure 1).[2]

The rosette-shaped colonies formed by *S. rosetta* resemble early stage morula embryos of diverse animals and develop through a process of incomplete cytokinesis from a single founding cell.[4] The induction of rosette development requires a bacterially produced signal from its prey *Algoriphagus machipongonensis*.[5] In a previous publication we identified the planar structure of the first rosette-inducing factor (RIF-1, **1**), and we demonstrated its extraordinary femtomolar potency.[6] In this report, we describe a modular total synthesis that defines the three-dimensional structure of RIF-1, the isolation of some naturally occurring analogs, and a rosette-inducing assay to establish initial structure−activity relations. In addition, we note that synthetic RIF-1 by itself does not completely recapitulate the activity of RIF-1 isolated from bacterial extract.

RIF-1 belongs to the small and poorly explored class of sulfonolipids.[7] Sulfonolipids have been reported as constituents of the cell envelopes of Bacteroidetes bacteria and are thought to contribute to the gliding motility frequently found in this group.[8] Sulfonolipids (**2**−**4**) closely resemble sphingolipids, such as (dihydro)ceramides (Figure 2, **5**), that are important membrane components in eukaryotes and act as both structural components and signaling molecules for cell death, survival, differentiation, and migration.[9] Sphingolipids and sulfonolipids have been reported rarely in bacteria and so far have only been isolated from the Bacteroidetes phylum and *Sphingomonas* genus, where their biological functions are poorly understood.[10] Both are amides of a fatty acid and an amine base called either sphingosine (for sphingolipids) or capnine (for sulfonolipids). Whereas sphingosine originates in the condensation of serine with a fatty acid followed by reduction and dehydrogenation, labeling studies with deuterated amino acids suggest that the capnine base is biosynthesized via the condensation of a fatty acyl-CoA with cysteic acid.[11]

To elucidate the stereochemistry of RIF-1 (Figure 2, **1**), we designed a flexible synthetic approach so that multiple derivatives of RIF-1 could be produced without changing the

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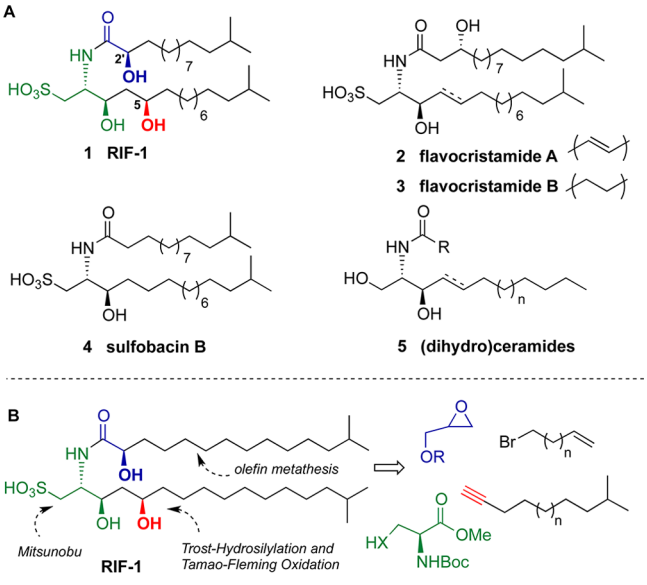
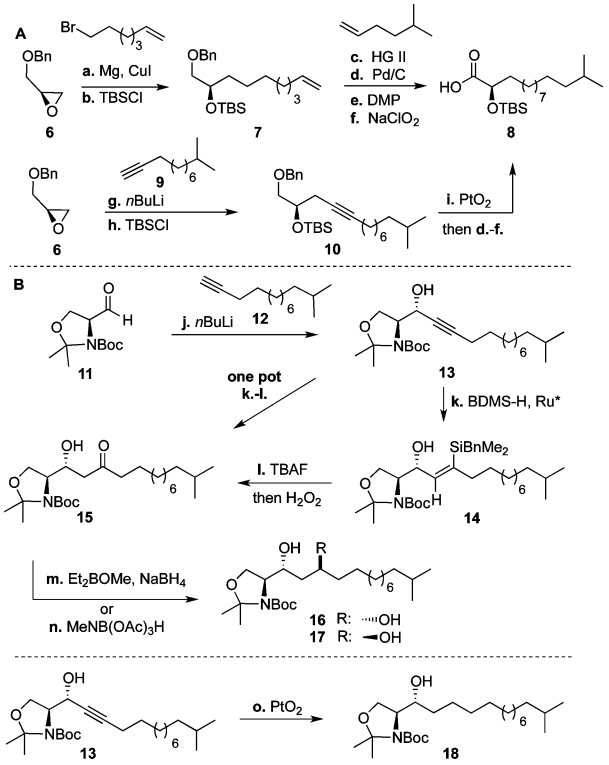


Figure 2. (A) RIF-1 and known sulfonolipids; (B) retrosynthesis of RIF-1.

main synthetic route. The absolute stereochemistry of RIF-1 was unknown, but we assumed it to be homologous to reported sulfonolipids (**2**–**4**).[7,12] Therefore, we first focused on the two completely unknown stereocenters at C-2′ and C-5, which generates four possible diastereoisomeric targets.

The synthesis of the α-hydroxy fatty acid commenced with the addition of cuprate reagent to benzyl-protected *R*(−) or *S*(+)-glycidol (Scheme 1).[13] After TBS protection, chain elongation was pursued by metathesis reaction with a second generation Hoveyda-Grubbs catalyst. A metathesis reaction at this point allowed access to other fatty acid precursors with different chain length and substitution pattern. The newly generated double bond and the Bn-protecting group were removed with Pd/C under hydrogen atmosphere in one step. The primary alcohol was then treated with Dess-Martin reagent and directly oxidized under standard reaction conditions with $NaClO_2$ in the presence of 2-methyl-2-butene yielding the desired α-hydroxy fatty acid **8**. The alternative fatty acid precursor **10** could be obtained by addition of alkyne **9** to glycidol ether **6**, subsequent TBS protection of the secondary alcohol, and reduction of the triple bond with PtO_2. Alkynes like **9** were synthesized according to literature procedures.[14] To assemble the sphingosine/capnine moiety, alkyne **12** was treated with *n*BuLi and reacted with Garner's aldehyde **11** in the presence of HMPA to yield compound **13** in acceptable 75% (*syn*:*anti* 20:80) yield (Scheme 1).[15] Over the course of the synthesis, it became apparent that a late stage Mitsunobu reaction for the introduction of the sulfonic acid group was a more attractive synthetic approach than using a cysteine-derived Garner's aldehyde as described by Takikawa et al.[12d] The next step involved a hydrosilylation reaction of **13** with benzyldimethylsilane (BDMS-H) as reported by Trost.[16] The reaction proceeded with excellent regiocontrol (>95:5) and afforded the desired silylated compound **14** in 90% yield. Subsequent Tamao-Fleming oxidation using TBAF and H_2O_2 (2 h) yielded ketone **15**. Gratifyingly, a one-pot procedure as reported by Trost starting from compound **13** could be accomplished affording ketone **15** in slightly higher overall yield (82%). Finally, β-hydroxy ketone was stereoselectively reduced using either Et_2BOMe as chelating reagent to give almost

Scheme 1. Representative Synthesis of (A) α-Hydroxy Acid and (B) Precursor of Capnine Base[a]



[a]Conditions: (a) Mg, CuI, THF, −20 °C, 84%; (b) TBSCl, TEA, DMAP, DMF, quant.; (c) 5-methyl-1-hexene, 5 mol % Hoveyda-Grubbs II catalyst, CH_2Cl_2, 40 °C, then; (d) Pd/C, H_2, EtOAc:EtOH 1:1, 2 d, 79% over 2 steps; (e) DMP, 30 mol % $NaHCO_3$, CH_2Cl_2, 0 °C → RT; then (f) $NaClO_2$, 2-methyl-butene, THF:BuOH:H_2O (3:1:1), RT, 3 h, 65% over 2 steps; (g) *n*BuLi, HMPA, THF, −78 °C, 82%; (h) TBSCl, TEA, DMAP, DMF, quant.; (i) PtO_2, H_2, EtOAc, 1 d, quant.; (j) *n*BuLi, HMPA, THF, −78 °C, 75% (*syn*:*anti* 20:80); (k) $[Cp^*Ru(NCCH_3)_3]^+PF_6^-$, BDMS-H, acetone, 0 °C → RT, 1 h, 90%; (l) TBAF, THF, 15 min, 0 °C; then H_2O_2, MeOH, K_2CO_3, 12 h, RT, 87%; (m) Et_2BOMe, $NaBH_4$, THF:MeOH 4:1, 77% (*syn*:*anti* > 90:10); (n) $Me_4NB(OAc)_3H$, MeOH:AcOH, −40 °C, 91% (*syn*:*anti* 20:80); (o) PtO_2, H_2, EtOAc, 1 d, quant.

exclusively *syn*-diol **16** in 77% (dr, *syn*:*anti* > 90:10),[17] or $Me_4NB(OAc)_3H$ to furnish *anti*-diol **17** with lower but satisfactory diastereoselectivity.[18] In addition, the alkyne moiety of **13** was hydrogenated using PtO_2 in nearly quantitative yield.

Since the stereochemistries of RIF-1's hydroxy groups at C-2′ and C-5 were unknown, we first continued our synthetic approach with α-hydroxy fatty acid **8** and *syn*-diol **16**. The cyclic isopropylaminal and Boc protecting groups were removed in 6 N HCl at 60 °C yielding free sphingoid base **19**, which was suitable for condensation with a fatty acid (Scheme 2).

The sphingolipid core structure **20** was assembled by treatment of **19** and fatty acid **8** with peptide coupling reagent EDAC (Scheme 2). Subsequent protection with TBSOTf and selective deprotection with TFA of the primary alcohol yielded the key precursor for RIF-1. Finally, a Mitsunobu reaction of **20** with thioacetic acid, one-pot deprotection and oxidation of the primary thiol with H_2O_2 afforded sulfonolipid **1** in an overall yield of 8% (9 steps) starting from Garner's aldehyde **11**. The spectroscopic data of **1** were in full agreement with the reported

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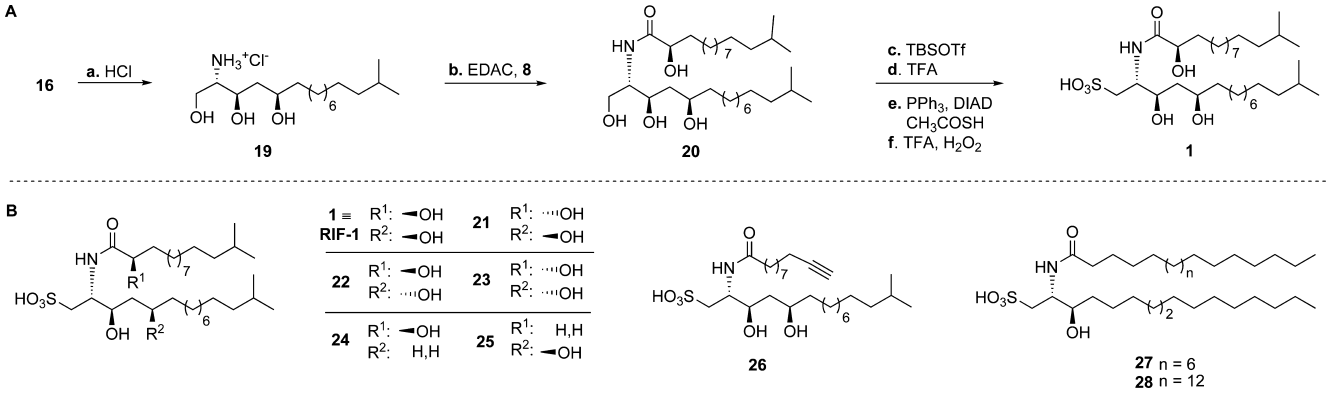
Scheme 2. (A) Completion of the Total Synthesis and (B) Synthesis of Structurally Related Sulfonolipids[a]



[a]Conditions: (a) 6 N HCl, MeOH, 60 °C, 6 h; then (b) EDAC, compound **8**, CH_2Cl_2, 2 h, 58% over 2 steps; (c) TBSOTf, 2,6-lutidine, DMAP, CH_2Cl_2, 89%; (d) 10% TFA in H_2O, THF, 0 °C → RT, 6 h, 58% + 30% sm; (e) PPh_3, DIAD, CH_2Cl_2, 1 h, 0 °C, then CH_3COSH, 81%; (f) TFA, H_2O_2, 4 h, RT, 65%.

data on natural RIF-1.[6] For sulfonolipids **21**–**28** an analogous synthetic route was performed.[19]

In a complementary approach we also investigated the diversity of sulfonolipids produced by *A. machipongonensis*.[5,6] The already reported sulfonolipids flavochristamide A and B (**2**, **3**) were only detected in negligible amounts by LC-MS. However, sulfobacin B (**4**) turned out to be one of the major sulfonolipid products under our standard growth condition.[19]

By detailed analysis of the lipid extracts we were able to isolate and characterize four unknown sulfonolipids **24**, **29**–**31**, which we named accordingly (sulfobacins C–F, Figure 3A). Synthetic compound **24**, missing the C-5 hydroxy group, had identical spectroscopic data as isolated sulfonolipid sulfobacin D, suggesting the depicted absolute stereochemistry. These synthetic and isolated materials allowed a preliminary structure–activity analysis for rosette induction. All synthesized (**1**, **21**–**28**) and isolated sulfonolipids (**1**, **4**, **24**, **29**–**31**) as well as sphingolipid intermediates of type **20** were tested over a broad concentration range (μM to fM) in a robust rosette colony-induction assay with the *S. rosetta* RCA cell line.[19] We also tested the corresponding capnine bases (**32**–**34**), which were obtained by hydrolysis with methanolic HCl. However, RIF-1 diastereomers (**21**–**23**), RIF-1 analogs (**24**–**31**), and capnine bases (**32**–**34**) did not induce rosette formation in *S. rosetta*. Small amounts of isomers of sulfonolipids **24** and **31** were also tested, but they too showed no rosette-inducing activity.[19] Only synthetic and natural RIF-1 (**1**) stimulated the development of solitary slow swimmers into rosette colonies. This unexpectedly restricted set of structural requirements indicates a highly specific substrate–receptor interaction. Synthetic RIF-1 does not completely replicate the biological activity of RIF-1 isolated from *A. machipongonensis* as shown by quantitative comparison (Figure 3B). We are currently exploring the reasons for this discrepancy by investigating additional bacterially produced molecules with rosette-inducing activity, molecules that synergize with RIF-1, and methods of delivering these highly hydrophobic signals.

In summary, we have defined the three-dimensional structure of RIF-1 through a modular total synthesis, characterized four new naturally occurring sulfonolipids, established the tight structural requirements for RIF-1's biological activity, and discovered that signals beyond RIF-1 may be needed for full activity.

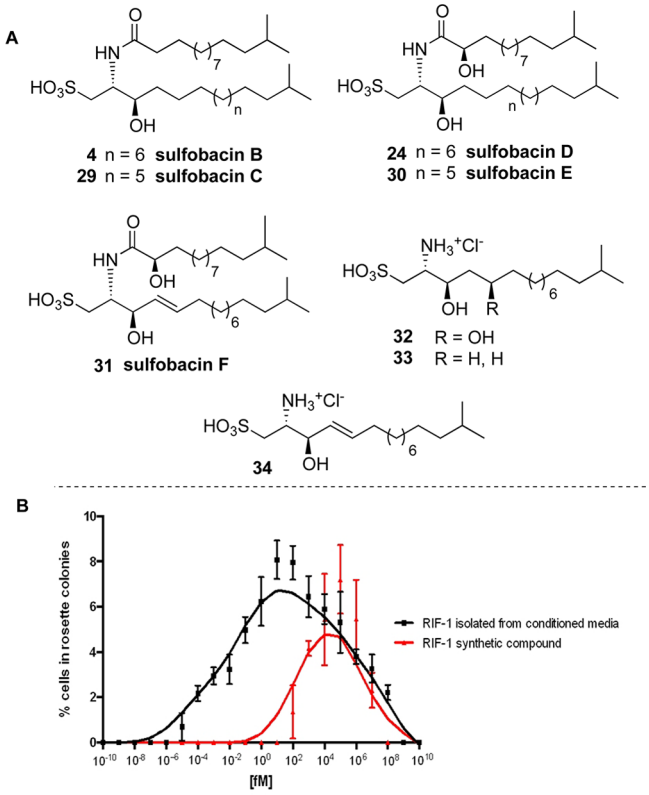


Figure 3. (A) Isolated sulfonolipids from *A. machipongonensis*, and corresponding capnine bases; and (B) dose–response curve of *S. rosetta* (fM concentration range) after treatment with natural isolate RIF-1 (black) and synthetic RIF-1 (red); error bars indicate standard deviation.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Syntheses, isolation procedures, compound characterization, and assay data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

jon_clardy@hms.harvard.edu; nking@berkeley.edu

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Present Addresses

[§]Leibniz Institute for Natural Product Research and Infection Biology e.V., Hans-Knöll-Institute (HKI), Beutenbergstrasse 11a, 07745 Jena, Germany.

[‖]Center for Microbial Oceanography Research and Education, 1950 East-west Rd., Honolulu, HI 96822.

Notes

The authors declare no competing financial interest.

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